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## **Development of a real-time RT-PCR assay that detects a broad range of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 subtypes**

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Porcine Reproductive and Respiratory Syndrome is a very important pig disease worldwide. The causative agent for the syndrome is a small enveloped positive-sense RNA virus (PRRSV) of approx. 15 kb. There is a high genetic and antigenic diversity among PRRSV isolates and isolates are divided into two genotypes designated Type 1 and Type 2. The Type 1 viruses can further be divided into subtypes, where subtypes 1, 2, and 3 have been acknowledged. Type 1 subtype 1 is found globally, whereas subtypes 2 and 3, so far, have only been detected in Eastern Europe and Russia. Clinical and experimental data suggest that infection with Type 1 subtype 2 and 3 cause more severe disease in pigs than Type 1 subtype 1. The movement of pigs between countries increases the risk of spread of subtypes 2 and 3 outside Eastern Europe and therefore it is very important that the diagnostic tools are able to detect all subtypes in order to provide the correct treatment and control. Based on in silico analyses of published primer sequences, most of the existing real-time RT-PCR assays for detection of PRRSV do not recognize all PRRSV Type 1 subtypes.

The aim of the study was to develop a real-time RT-PCR assay capable of detecting a broad range of PRRSV Type 1 subtype 1, 2, 3 and isolates not allocated to subtypes (atypical Type 1 strains) with a high PCR efficiency and good range of quantification.

For the design of the real-time RT-PCR assay five (5) complete genomes from viruses representing all Type 1 subtypes 1, 2, and 3 were used. The five complete genomes were aligned and a dual labeled probe chemistry assay was designed at a conserved region of the genome (ORF2 coding region). The assay was validated on RNA extracted from cell culture supernatant or serum from samples representing all subtypes and one of the atypical Type 1 strains.

The assay recognized all tested strains with PCR efficiencies in the range 90-99% and a range of quantification of four-five 10-fold dilution steps.

Surveillance programs of PRRSV are in most countries based on serology, but most of the applied serological tests cannot discriminate between genotypes or subtypes. Thus, to be effective, PRRSV monitoring programs must be based on the use of sensitive real-time RT-PCR tests that recognize a broad range of PRRSV isolates. The presented assay provides laboratories with a new assay.